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(54) PREVENTIVE AND/OR THERAPEUTIC AGENT FOR KIDNEY DISEASES

(57) A preventive and/or therapeutic agent for kidney diseases, containing as the active ingredient a tumor cytotoxic factor II (TCF-II) mutant which has undergone point mutation. The mutant is TCF-II wherein the Arg-Lys-Arg-Arg amino acid sequence beginning at the second position from the N-terminal is replaced by Ala-Ala-Ala-Ala or the Lys-Ile-Lys-Thr-Lys-Lys amino acid sequence beginning at the 27th position therefrom is replaced by Ala-Ile-Ala-Thr-Ala-Ala.

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Description

Technological field

[0001] The present invention relates to an agent for preventing and/or treating renal disease comprising point mutant TCF-II mutant as an effective ingredient. The agent for preventing and/or treating renal disease of the present invention is useful for preventing and/or treating renal diseases such as chronic nephropathy related with ischemic renal disorder, drug-induced renal disorder, diabetic nephropathy, glomerular nephropathy, glomerulosclerosis, membranous nephropathy, autoimmune disease and nephrose or renal insufficiency caused by the above.

Background technology

[0002] Any effective therapeutic agent for renal diseases such as chronic nephropathy related with ischemic renal disorder, drug-induced renal disorder, diabetic nephropathy, glomerular nephropathy, glomerulosclerosis, membranous nephropathy, autoimmune disease and nephrose or renal insufficiency caused by the above has not been found so far. In a clinical practice, only maintenance therapy, that is, removal of derangement by dialysis, management of nutrition or administration of diuretic or cardiac or steroid therapy is carried out considering symptoms. Therefore, an effective drug in renal diseases is eagerly desired.

[0003] TCF-II is a glycoprotein (WO 90/10651) found by the present inventors which is known as Tumor Necrosis Factor produced by human fibroblast IMR-90 and has excellent pharmacological activities such as an activity of proliferating hepatocyte, an activity of proliferating renal cell, an anti-tumor activity and so on. Naturally occurring TCF-II and recombinant TCF-II are known. Further, a mutant protein without carbohydrate chain and a point mutant TCF-II (WO 96/20214) are also known.

Disclosure of the invention

[0004] Considering the above situations, the present inventors eagerly investigated to look for an effective substance for these renal diseases and found that TCF-II mutant, especially, a TCF-II mutant which is a point mutant of amino acid sequence at the second from N-terminal, that is, from Arg-Lys-Arg-Arg to Ala-Ala-Ala-Ala or another TCF-II mutant whose amino acid sequence at 27 th from N-terminal was changed into Ala-Ile-Ala-Thr-Ala-Ala from Lys-Ile-Lys-Thr-Lys-Lys, was effective for preventing and/or treating renal diseases. Accordingly, an object of the present invention is to provide a novel agent for preventing and/or treating renal diseases comprising TCF-II mutant as an effective ingredient.

[0005] The present invention relates to an agent for preventing and/or treating renal diseases comprising TCF-II mutant, especially, a TCF-II mutant which is a point mutant of amino acid sequence at the second from N-terminal, that is, from Arg-Lys-Arg-Arg to Ala-Ala-Ala-Ala or another TCF-II mutant whose amino acid sequence at 27 th from N-terminal was changed to Ala-Ile-Ala-Thr-Ala-Ala from Lys-Ile-Lys-Thr-Lys-Lys, as an effective ingredient. The agent for preventing and/or treating renal disease of the present invention is useful for preventing and/or treating renal diseases such as chronic nephropathy related with ischemic renal disorder, drug-induced renal disorder, diabetic nephropathy, glomerular nephropathy, glomerulosclerosis, membranous nephropathy, autoimmune disease and nephrose or renal insufficiency caused by the above.

[0006] Point mutant TCF-II of an effective ingredient of the present invention can be prepared by synthesizing oligonucleotide substituted with corresponding base sequence to mutation site of TCF-II mutant, followed by site-directed mutagenesis using TCF-II cDNA as a template by polymerase chain reaction (PCR) method. cDNA obtained as above can be inserted into a vector having an appropriate expression promoter (Cytomegalovirus (CMV) SRα (Mol. Cell. Biol. vol.8, No.1 pp466-472 (1988)) and Japanese unexamined laid-open patent application No. 277489 (1991)), followed by transfection thereof into eukariotic cell such as mammlian cell. TCF-II mutant desired can be prepared by recovering it from culture medium of the culture of the above transfected cell. As TCF-II mutant used in the present invention, any TCF-II with an artificial mutation can be used but, more preferably, a TCF-II mutant whose amino acid sequence at the second from N-terminal, was changed from Arg-Lys-Arg-Arg to Ala-Ala-Ala-Ala or another TCF-II mutant whose wino acid sequence at 27th from N-terminal was changed to Ala-Ile-Ala-Thr-Ala-Ala from Lys-Ile-Lys-Thr-Lys-Lys (these mutants were described in WO 96/20214) can be used.

[0007] The agent for preventing and/or treating renal diseases of the present invention can be administered intravenously, intra muscularly or subcutaneously as injections. This pharmaceutical preparation can be manufactured according to a known method of manufacturing pharmaceutical preparation and , if necessary, a pH conditioner, buffer, stabilizer etc. can be added thereto. Dose of the pharmaceutical preparation of the present invention can vary depending on degree of severeness of symptom, health conditions, age, body weight and will not be limited, but for an adult person per day pharmaceutical preparation containing 0.6 mg-600 mg of TCF-II, preferably 6 mg-60 mg, can be administered once or more per day.

Brief description of the drawings

[0008]

Figure 1 shows defensive effect of TCF-II mutant

(RKRR2AAAA) against death caused by renal insufficiency induced with mercuric chloride in example 3.

Figure 2 shows defensive effect of TCF-II mutant (KIKTKK27AIATAA) against death caused by renal insufficiency induced with mercuric chloride in example 3.

Best embodiment for practice of the invention

[0009] The present invention will be described in more detail. However, these are only exemplification and will not limit the present invention.

[Example 1]

Preparation of TCF-II mutant

[0010] According to a method described in WO 96/20214, two species of point mutant, that is, a TCF-II mutant whose amino acid sequence at the second from N-terminal was changed from Arg-Lys-Arg-Arg to Ala-Ala-Ala-Ala (hereinafter referred to RKRR2AAAA) and another TCF-II mutant whose amino acid sequence at 27th from N-terminal was changed from Lys-Ile-Lys-Thr-Lys-Lys to Ala-Ile-Ala-Thr-Ala-Ala (hereinafter referred to KIKTKK27AIATAA) were prepared. That is, shaking culture of E.coli comprising an expression vector of RKRR2AIAA cDNA (FERM BP-5266) and E col. comprising an expression vector of KIKTKK27AIATAA cDNA was carried out in L-medium (400 ml) containing 50 μg/ml ampicillin at 37°C. When OD 600 became 1.0, spectinomycin (Sigma) was added so that the final concentration thereof would be 0.3 mg/ml, and the culture medium was cultured overnight. According to the method of Maniatis (Molecular Cloning 2nd ed. pp1.21-1. 52 (1989), Cold Spring Harbor Laboratory), plasmid DNA was separated by alkaline SDS method and the expression plasmodia of each mutant was purified by cesium chloride density gradient centrifugation.

[0011] These obtained expression plasmodia (200 μg) were introduced into CHO cell. The expression plasmodia (200 μg) and expression plasmodia of pSV2 of blastsidine resistant gene (10 μg/Funakoshi) DNA which were dissolved in TE (10 mM Tris-HCl (pH 8.0)-1 mMEDTA) in advance, were transfected into 2 x 10^6 CHO cells suspended in 0.8 ml of IMDM culture medium (Gibco) containing 10% calf fetal serum by electroporation After electroporation carried out under the conditions of 330 V and 960 μF, cell suspension was left at room temperature for 10 minutes, suspended in 10 ml IMDM culture medium containing 10% calf fetal serum and cultured in a CO_2 incubator (5% CO_2) at 37°C for 2 days. After 2 days since then, cells were deprived from the bottom of flask by tripsin (Gibco) treatment and the number of viable cells was counted and cells were disseminated in 96-well plate (Nunc) so as to be 10,000 cells/well, which was cultured in 200 μl selected medium/well containing 5 μg/ml blastosidine (Funakoshi) at 37°C in CO_2 incubator (5% CO_2). After 2-3 weeks, 50 μl of culture supernatant was taken from each well and, by enzyme-immuno-assay thereof (N. Shima et. al., Gastropenterologia Japonica, vol. 26, No. 4, pp477-482 (1991)), cells producing TCF-II mutant were selected TCF-II mutant producing cells were cultured in 50-200 flasks (each volume is 225 cm^2) containing 100 ml of culture medium at 37°C in a CO_2 incubator (5% CO_2) for 4-7 days and 5-20 L of cultured supernatant was recovered. Each mutant was purified from the above culture supernatant using Heparin-Sepharose CL-6B column (25 mm x 120 mm, Pharmacia), Mono S column (5 mm x 50 mm Pharmacia) and Heparin 5-PW column (8 mm x 75 mm, Toso). Obtained TCF-II mutant was dialyzed against phosphate buffer solution (PBS) containing 0.01% Tween 20 to be the final product. The protein determination of the final product was carried out by lowery method and the purity thereof was examined by SDS electrophoresis and, then, amino acid sequencer thereof confirmed amino acid sequence.

[Example 2]

Manufacturing of pharmaceutical preparation of TCF-II

[0012] An example of manufacturing injections of recombinant TCF-II obtained in example 1 was shown.

(1)	TCF-II Mutant	20 μg
	human serum albumin	100 mg

[0013] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization

(2)	TCF-II Mutant	20 μg
	Tween 80	1 mg
	human serum albumin	100 mg

[0014] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization

(3)	TCF-II Mutant	20 μg
	Tween 80	2 mg
	Sorbitol	4 g

[0015] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(4)	TCF-II Mutant	20 μg
	Tween 80	1 mg
	Glycine	2 g

[0016] The above composition was dissolved in physiological saline solution foe injections so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(5)	TCF-II Mutant	20 μg
	Tween 80	1 mg
	Sorbitol	2 g
	Glycine	1 g

[0017] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(6)	TCF-II Mutant	20 μg
	Sorbitol	4 g
	human serum albumin	50 mg

[0018] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(7)	TCF-II Mutant	20 μg
	Glycine	2 g
	human serum albumin	50 mg

[0019] The above composition was dissolved in physiological saline solution for injections so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

(8)	TCF-II Mutant	20 μg
	human serum albumin	50 mg

[0020] The above composition was dissolved in citric acid buffer solution with pH 6.03 so that the total volume would be 20ml. Then it was divided into vials containing 2 ml each after sterilization and sealed after lyophilization.

[0021] These lyophilized products will be dissolved in sterilized distilled water on use.

[Example 3]

Defensive effect against death caused by renal insufficiency induced with mercuric chloride.

[0022] Defensive effect of TCF-II mutant against death caused by renal insufficiency induced with mercuric chloride was examined using RKRR2AAAA mutated at the second amino acid from N-terminal of TCF-II RKRR2AAAA in which the amino acid residues from the N-terminal of TCF-II and KIKTKK27AIATAA in which the amino acid residues from the 27th amino acid, which were obtained in example 1.

[0023] That is, one of TCF-II mutants (25μg/mouse/time), TCF-II as positive control (25, 50, 100 μg/mouse/time), or vehicle was administered intravenously to male ICR mice (body weight:30-35 g; n= 20 per group) twice daily (total 9 times). At 6 hours after the final administration, 5 mg/kg mercuric chloride (Wako-junyaku) was administered intravenously, and the survival of mice was monitored to examine the protective effect of TCF-II mutant on mortality. The results are shown in figure 1 and figure 2. From the results, the TCF-II treatment and TCF-II mutant treatment apparently protected the mortality caused by mercuric chloride - induced renal failure, compared to the vehicle treatment. Furthermore, the activities of TCF-II mutants were more potent than that of TCF-II (RKRR2AAAA was four times and KIKTKK27AIATAA was two times as

potent as native TCF-II).

Industrial applicability

[0024] The present invention provides an agent for preventing and/or treating renal diseases.

[0025] The present invention is useful for preventing and/or treating renal diseases such as chronic nephropathy related with ischemic renal disorder, drug-induced renal disorder, diabetic nephropathy, glomerular nephropathy, glomerulosclerosis, membranous nephropathy, autoimmune disease and nephrose or renal insufficiency caused by the above, comprising TCF-II mutants, that is, a TCF-II mutant in which amino acid sequence at the second from N-terminal of TCF-II was changed from Arg-Lys-Arg-Arg to Ala-Ala-Ala-Ala and another TCF-II mutant in which amino acid sequence at the 27th from the N-terminus was changed from Lys-Ile-Lys-Thr-Lys-Lys to Ala-Ile-Ala-Thr-Ala-Ala as an effective ingredient.

Reference of Microorganism

[0026]

1) Organization of Deposition

National Institute of Bioscience and Human-Technology,

Agency of Industrial Science and Technology,

Ministry of International Trade and Industry
Address 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan
Date of Deposition: November 10, 1994
(The microorganism was originally deposited above of November 10, 1994, and transferred to the deposit based on the Treaty on October 25, 1995)
Accession Number: FERM BP-5265

2) Organization of Deposition

National Institute of Bioscience and Human-Technology,

Agency of Industrial Science and Technology,

Ministry of International Trade and Industry
Address: 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan
Date of Deposition: November 10, 1994
(The microorganism was originally deposited above of November 10, 1994, and transferred to the deposit based on the Treaty on October 25, 1995)
Accession Number: FERM BP-5266

Claims

1. An agent for preventing and/or treating renal disease comprising a TCF-II mutant with point mutations.

2. An agent for preventing and/or treating renal disease according to claim 1 said mutant TCF-II is TCF-II mutant in which amino acid sequence at the second from the N-terminal of TCF-II was changed from Arg-Lys-Arg-Arg to Ala-Ala-Ala-Ala as an effective ingredient.

3. An agent for preventing and/or treating renal disease according to claim 1 said point mutant TCF-II is TCF-II mutant in which amino acid sequence at the 27th from the N-terminal of TCF-II was changed from Lys-Ile-Lys-Thr-Lys-Lys to Ala-Ile-Ala-Thr-Ala-Ala as an effective ingredient.

4. The agent for preventing and/or treating renal diseases according to anyone of claim 1 to claim 3 wherein said renal disease is renal insufficiency.

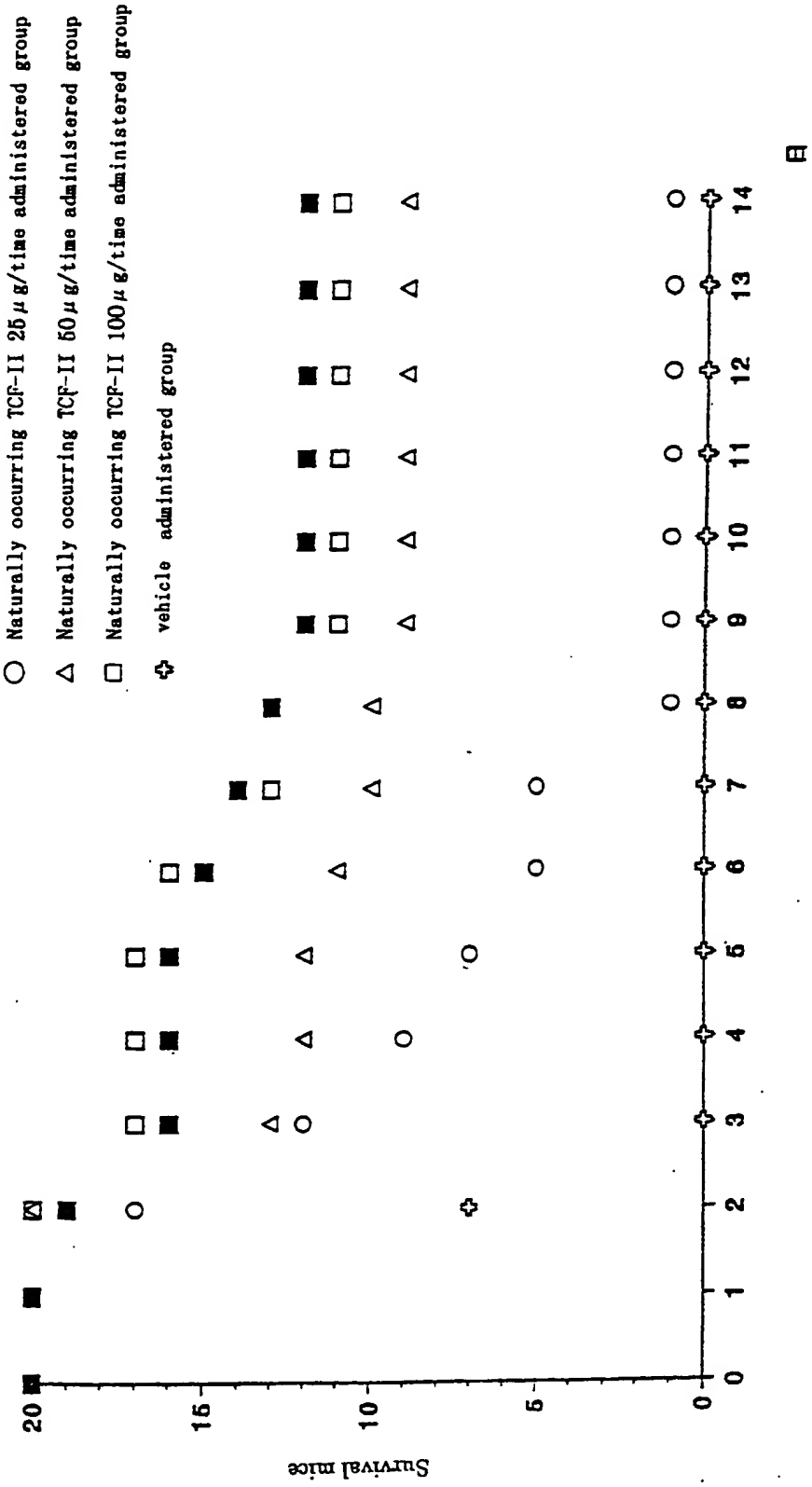
TCF-II mutant (RKRR2AAA) 25 μg/time administered group
Naturally occurring TCF-II 25 μg/time administered group
Naturally occurring TCF-II 50 μg/time administered group
Naturally occurring TCF-II 100 μg/time administered group
vehicle administered group
Survival mice
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
日

Fig. 1

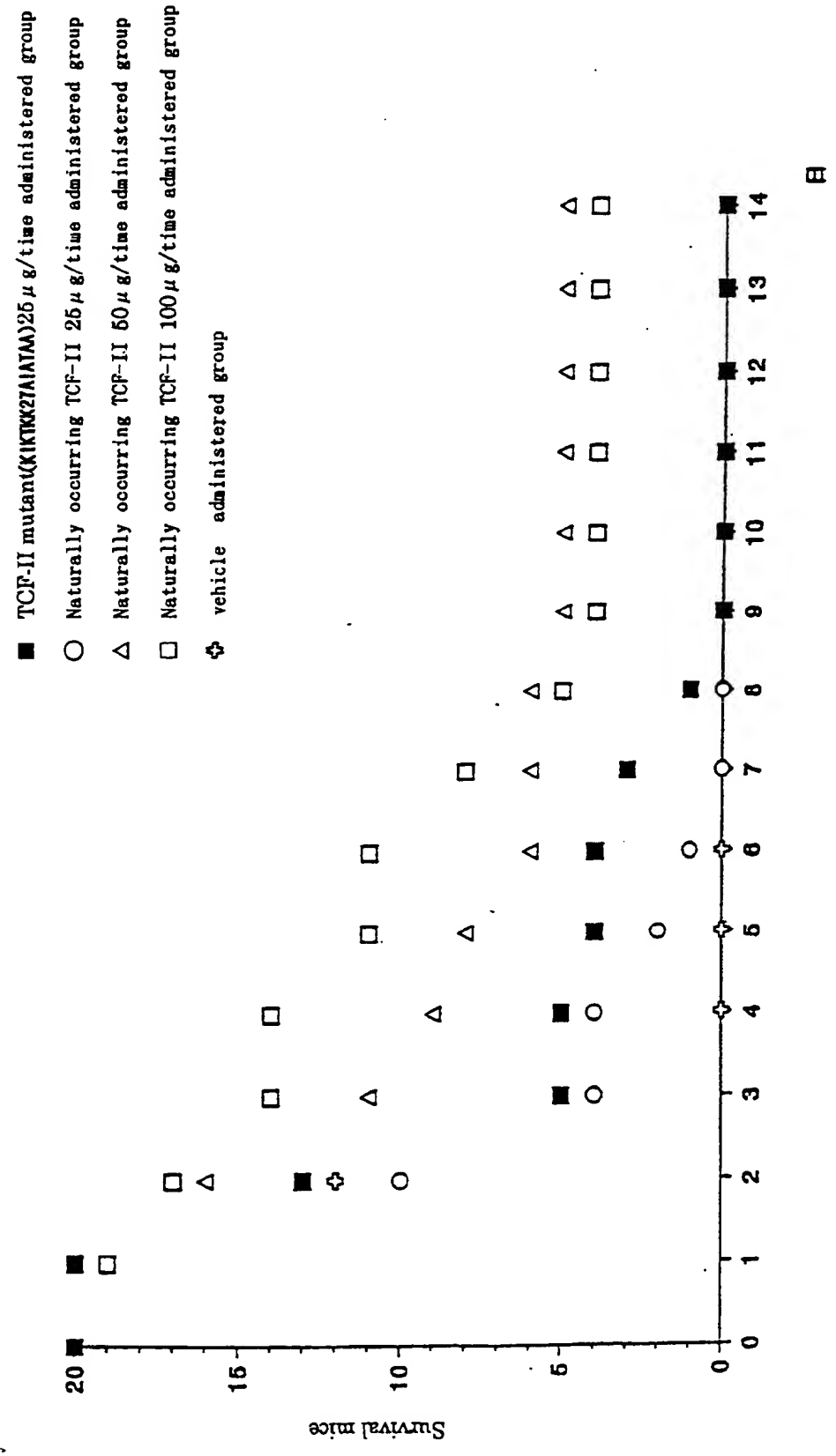
■ TCF-II mutant(K1K7K27A1A7A)25μg/time administered group
○ Naturally occurring TCF-II 25μg/time administered group
△ Naturally occurring TCF-II 50μg/time administered group
□ Naturally occurring TCF-II 100μg/time administered group
✥ vehicle administered group
Survival mice
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
日

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP98/01221

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[6] A61K38/22, C07K14/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[6] A61K38/22, C07K14/52

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE (STN), CAPLUS (STN), WPIDS (STN), WPI (DIALOG)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, 96/20214, A1 (Snow Brand Milk Products Co., Ltd.), July 4, 1996 (04. 07. 96), Claim 5 & EP, 757994, A1 & NZ, 298142, A	1-4
Y	Toyohiro Takehara et al., "Structure of Hapatic Cell Growth Factor (HGF) and Physiological Effect (in Japanese)", Protein, Nucleic Acid and Enzyne, 1991, Vol. 36, No. 7, pp.265-274	1-4
Y	JP, 6-40935, A (Snow Brand Milk Products Co., Ltd.), February 15, 1994 (15. 02. 94),	4
A	Column 1 & EP, 588477, A2 & AU, 9341945, A	1-3
A	JP, 64-68400, A (Sapporo Breweries Ltd.), March 14, 1989 (14. 03. 89) (Family: none)	1-4

[x] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
May 18, 1998 (18. 05. 98)	May 26, 1998 (26. 05. 98)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP98/01221

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Nobuyuki Shima et al., "Structure of Tumor Cell Damage Factor (F-TCF) and Various Bioactivity (in Japanese)", The Japanese Journal of Clinical Medicine, 1992, Vol. 50, No. 8, pp.270-274	1-4

Form PCT/ISA/210 (continuation of second sheet) (July 1992)